

**Amendments to the Claims:**

This listing of claims will replace all prior versions, and listings of claims in the application:

**Listing of Claims:**

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52. (new) A method for determining an unknown starting quantity of a target nucleic acid sequence in a test sample, the method comprising the steps of:
- a) amplifying the unknown starting quantity of the target nucleic acid sequence in the test sample;
  - b) amplifying a plurality of known starting quantities of a calibration nucleic acid sequence in respective calibration samples;
  - c) determining a respective threshold value for each of the known starting quantities of the calibration nucleic acid sequence in the calibration samples and for the target nucleic acid sequence in the test sample, wherein the threshold value is determined for each nucleic acid sequence in a respective sample by:
    - i) measuring, at a plurality of different times during amplification, at least one signal whose intensity is related to the quantity of the nucleic acid sequence being amplified in the sample;
    - ii) storing signal values defining a growth curve for the nucleic acid sequence;
    - iii) calculating a derivative of the growth curve;
    - iv) identifying a positive peak of the derivative;
    - v) determining if the positive peak exceeds a threshold level; and
    - vi) if the positive peak exceeds the threshold level, then calculating the threshold value corresponding to the positive peak of the derivative;
  - d) deriving a calibration curve from the threshold values determined for the known starting quantities of the calibration nucleic acid sequence in the calibration samples; and
  - e) determining the starting quantity of the target nucleic acid sequence in the test sample using the calibration curve and the threshold value determined for the target sequence.
53. (new) The method of claim 52, wherein each of the threshold values comprises a threshold cycle number.

54. (new) The method of claim 52, wherein each of the threshold values comprises an elapsed time of amplification.
55. (new) The method of claim 52, wherein the threshold level is a noise-based threshold level.
56. (new) The method of claim 52, wherein the threshold level is a user-defined threshold level.
57. (new) The method of claim 52, wherein the second derivative of the growth curve is calculated, and the positive peak of the second derivative is identified.
58. (new) The method of claim 52, wherein the first derivative of the growth curve is calculated, and the positive peak of the first derivative is identified.
59. (new) The method of claim 52, wherein the step of calculating a derivative of the growth curve comprises calculating second derivative values of the growth curve at a number of different measurement points in the reaction to yield a plurality of second derivative data points, and the step of calculating the threshold value corresponding to the positive peak comprises:
  - i) fitting a second order curve to the second derivative data points; and
  - ii) calculating the threshold value as the location of a peak of the second order curve.
60. (new) The method of claim 52, wherein the step of calculating a derivative of the growth curve comprises determining the second derivative of the growth curve with respect to cycle number, and wherein the threshold value is calculated as the location, in cycles, of the positive peak of the second derivative.

61. (new) The method of claim 52, wherein the step of calculating a derivative of the growth curve comprises determining the first derivative of the growth curve with respect to cycle number, and wherein the threshold value is calculated as the location, in cycles, of the positive peak of the first derivative.
62. (new) The method of claim 52, wherein the step of calculating a derivative of the growth curve comprises determining the second derivative of the growth curve with respect to time of amplification, and wherein the threshold value is calculated as the location, in time of amplification, of the positive peak of the second derivative.
63. (new) The method of claim 52, wherein the step of calculating a derivative of the growth curve comprises determining the first derivative of the growth curve with respect to time of amplification, and wherein the threshold value is calculated as the location, in time of amplification, of the positive peak of the first derivative.
64. (new) A method for determining an unknown starting quantity of a target nucleic acid sequence in a test sample, the method comprising the steps of:
  - a) amplifying the unknown starting quantity of the target nucleic acid sequence in the test sample;
  - b) amplifying a plurality of known starting quantities of a calibration nucleic acid sequence in respective calibration samples;
  - c) determining a respective threshold value for each of the known starting quantities of the calibration nucleic acid sequence in the calibration samples and for the target nucleic acid sequence in the test sample, wherein the threshold value is determined for each nucleic acid sequence in a respective sample by:
    - i) measuring, at a plurality of different times during amplification, at least one signal whose intensity is related to the quantity of the nucleic acid sequence being amplified in the sample;

- ii) storing signal values defining a growth curve for the nucleic acid sequence;
  - iii) calculating a second derivative of the growth curve;
  - iv) identifying a zero-crossing of the second derivative;
  - v) determining if the signal value defining the growth curve at the zero-crossing exceeds a threshold level; and
  - vi) if the signal value defining the growth curve at the zero-crossing exceeds the threshold level, then calculating the threshold value corresponding to the zero-crossing;
- d) deriving a calibration curve from the threshold values determined for the known starting quantities of the calibration nucleic acid sequence in the calibration samples; and
- e) determining the starting quantity of the target nucleic acid sequence in the test sample using the calibration curve and the threshold value determined for the target sequence.
65. (new) The method of claim 64, wherein each of the threshold values comprises a threshold cycle number.
66. (new) The method of claim 64, wherein each of the threshold values comprises an elapsed time of amplification.
67. (new) The method of claim 64, wherein the threshold level is a calculated, noise-based threshold level.
68. (new) The method of claim 64, wherein the threshold level is a user-defined threshold level.

69. (new) The method of claim 64, wherein the second derivative of the growth curve is calculated with respect to cycle number, and the threshold value is calculated as the location, in cycles, of the zero-crossing of the second derivative.
70. (new) The method of claim 64, wherein the step of calculating the second derivative of the growth curve comprises calculating second derivative values of the growth curve at a number of different measurement points to yield a plurality of second derivative data points, and wherein the threshold value corresponding to the zero-crossing is calculated by interpolation between at least two of the second derivative data points.
71. (new) The method of claim 64, wherein the second derivative of the growth curve is calculated with respect to time of amplification, and the threshold value is calculated as the location, in time of amplification, of the zero-crossing of the second derivative.
72. (new) A method for determining an unknown starting quantity of a target nucleic acid sequence in a test sample, the method comprising the steps of:
- a) amplifying the unknown starting quantity of the target nucleic acid sequence in the test sample;
  - b) amplifying a plurality of known starting quantities of a calibration nucleic acid sequence in respective calibration samples;
  - c) determining a respective threshold value for each of the known starting quantities of the calibration nucleic acid sequence in the calibration samples and for the target nucleic acid sequence in the test sample, wherein the threshold value is determined for each nucleic acid sequence in a respective sample by:
    - i) measuring, at a plurality of different times during amplification, at least one signal whose intensity is related to the quantity of the nucleic acid sequence being amplified in the sample;
    - ii) storing signal values defining a growth curve for the nucleic acid sequence;

- iii) calculating a second derivative of the growth curve;
  - iv) identifying a negative peak of the second derivative;
  - v) determining if the signal value defining the growth curve at the negative peak exceeds a threshold level; and
  - vi) if the signal value defining the growth curve at the negative peak exceeds the threshold level, then calculating the threshold value corresponding to the negative peak;
- d) deriving a calibration curve from the threshold values determined for the known starting quantities of the calibration nucleic acid sequence in the calibration samples; and
- e) determining the starting quantity of the target nucleic acid sequence in the test sample using the calibration curve and the threshold value determined for the target sequence.
73. (new) The method of claim 72, wherein each of the threshold values comprises a threshold cycle number.
74. (new) The method of claim 72, wherein each of the threshold values comprises an elapsed time of amplification.
75. (new) The method of claim 72, wherein the threshold level is a calculated, noise-based threshold level.
76. (new) The method of claim 72, wherein the threshold level is a user-defined threshold level.
77. (new) The method of claim 72, wherein the step of calculating the second derivative of the growth curve comprises calculating second derivative values of the growth curve at a number of different measurement points in the reaction to yield a plurality of second



derivative data points, and the step of calculating the threshold value corresponding to the negative peak comprises:

- i) fitting a second order curve to the second derivative data points; and
- ii) calculating the threshold value as the location of a negative peak of the second order curve.

78. (new) The method of claim 72, wherein the second derivative of the growth curve is calculated with respect to cycle number, and wherein the threshold value is calculated as the location, in cycles, of the negative peak of the second derivative.

79. (new) The method of claim 72, wherein the second derivative of the growth curve is calculated with respect to time of amplification, and wherein the threshold value is calculated as the location, in time of amplification, of the negative peak of the second derivative.